Objective: The emergence of resistance due to Metallo-b-Lactamases (MBLs) in Pseudomonas aeruginosa has become a therapeutic challenge. P. aeruginosa strains producing MBLs have been reported from various Asian countries which include Taiwan, India, Japan, China, Korea and Malaysia. The objective of this study is to investigate production of metallo-beta lactamases (MBLs) among clinical isolates of imipenem resistant P. aeruginosa.

Methods: Ninety clinical isolates of P. aeruginosa were obtained from the patients in University Malaya Medical Centre (UMMC) and minimum inhibitory concentration (MIC) was determined. MBL producing isolates were phenotypically identified using combined disk and double disk synergy tests. Duplex PCR and Restriction fragment length polymorphism (RFLP) analysis were carried out to determine the presence of MBL genes and to further confirm the classes of integrons respectively. In addition, Random amplification of polymorphic DNA (RAPD) analysis was performed to investigate clonality of the P. aeruginosa isolates.

Results: Of 90 clinical isolates, 69% and 31% were positive for combined disk and double disk synergy tests respectively. Duplex PCR assays detected 32 MBLs producers with 54.5% and 42.4% isolates identified as blaVIM and blaIMP types respectively. Sequence determination of the amplified MBL genes revealed the closest sequence similarities with blaIMP7 (12 isolates), blaIMP4 (2 isolates), blaVIM2 (17 isolates) and blaVIM11. The blaIMP,SPM,SIM MBL genes were not detected amongst the isolates tested. The MICs of MBLs producers to imipenem ranged from 96 256 μg/ml as compared to 8 32 μg/ml of non-MBL producers. It was also found the MBLs producers were significantly more resistant to ciprofloxacin, gentamicin, cefazidime, amikacin, netilmicin and cefoperazone than non-MBL producers. Restriction analysis showed that all the 32 MBL-genes positive isolates harboured class 1 Integron. Among the non-MBLs producers, 22 and 3 isolates were identified to have Class I and Class II integrons respectively. The RAPD analysis demonstrated 33 different patterns. Among MBL genes positive, 13 RAPD genotyped; Types 5 were predominant (7/32) and found to harbor the VIM gene. The 58 non-MBLs isolates had 20 RAPD types; Type 1 was the predominant (12/58).